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INTRACELLULAR RECORDINGS FROM GANGLIA OF THE THORACIC SYMPATHETIC CHAIN OF THE GUINEA-PIG

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SUMMARY

1. Intracellular recordings have been obtained from cells of thoracic paravertebral sympathetic ganglia of guinea-pigs.

2. Resting potentials were 50–70 mV. The steady-state current–voltage curve was linear for all hyperpolarizations and usually for depolarizations up to 10 mV. The slope of this curve was 19–186 MΩ (mean 55 MΩ). Time constants of cells were 6–14 msec (mean 9·1 msec).

3. Most cells maintained a high rate of firing of action potentials during strong current pulses.

4. Indirect stimulation via the rami communicantes or the interganglionic nerves of the sympathetic chain evoked a graded synaptic response which could lead to the discharge of one or more action potentials. Hexamethonium blocked the responses to such stimulation. Occasionally an all-or-none response remained, apparently conducted antidromically along the cell's axon.

5. Spontaneous synaptic potentials were observed. Their occurrence frequency was increased during indirect stimulation. If they are assumed to represent single quanta of release of transmitter, the quantal content of a threshold synaptic potential was 8–20 quanta.

6. Preganglionic fibres could ascend at least three segments or descend two after entering the sympathetic chain. They had conduction velocities of 0·1–1·1 m/sec.

7. Inexcitable cells were frequently impaled. Repetitive indirect stimulation gave rise in these cells to a large (20 mV) slow depolarizing response.

INTRODUCTION

Electrophysiological studies of mammalian paravertebral sympathetic ganglia have been confined chiefly to extracellular recordings from the superior cervical ganglion, and to a lesser extent, the stellate ganglion. Intracellular recordings have mostly been carried out on amphibian

ganglia, the only extensive mammalian studies being those on the superior cervical ganglion of the rabbit (Eccles, 1955, 1963; Erulkar & Woodward, 1967, 1968) and on isolated guinea-pig pelvic ganglia (Blackman & Holman, 1967; Blackman, Crowcroft, Devine, Holman & Yonemura, 1969). Other work has been briefly reported by Skok (1963), recording from stellate ganglia of cats, and Libet & Tosaka (1966), recording from isolated superior cervical ganglia of the rabbit.

The present paper describes the results of intracellular recordings from thoracic sympathetic ganglia of guinea-pigs, and investigates the properties and synaptic responses of the cells in these ganglia. In the course of the work it appeared that there were few electrophysiological studies of fibre pathways in the sympathetic chain: the evidence available comes from Langley's classic experiments (Langley, 1899), from studies on a lumbar ganglion (Obrador & Odoriz, 1936) and from degeneration studies (Foley & Schnitzlein, 1957) and neurological observations following partial sympathectomy (Boyd, 1957). It was therefore thought desirable to determine preganglionic fibre-pathways and patterns of innervation.

A preliminary report of some of this work has already been published (Purves & Blackman, 1968).

METHODS

Small guinea-pigs (150–250 g) were killed by a blow on the head. A 1–2 cm length of the right thoracic sympathetic chain (at the level T5–T9) was dissected free from the pleura and prevertebral musculature. This length included a variable number of ganglia together with as much as possible (about 0·5 cm) of the corresponding rami communicantes. Usually each ganglion had one ramus only, presumably of mixed character containing both pre- and post-ganglionic fibres. The preparation was pinned out in bath (Fig. 1) through which flowed a physiological salt solution of ionic composition (mM): Na 143·5, K 5·9, Ca 2·5, Mg 1·2, Cl 128·2, HCO_3 25·0, H_2PO_4 1·2, SO_4 1·2, glucose 11; the solution was warmed to 35–37° C, and bubbled continuously with 95% O_2, 5% CO_2 gas mixture.

The ramus communicans to one of the ganglia, and the interganglionic nerve above and below the ganglion were taken up into suction electrodes, providing three inputs to the ganglion for indirect stimulation. Stimuli (0·2 sec rectangular pulses) were supplied by two Grass Model S4C stimulators with SIU4A stimulus Isolation Units, and a Devices Isolated stimulator, all triggered by a Devices Digitimer. For recording, micro-electrodes filled with 3 M-KCl and having resistances of 30–70 MΩ were used. Signals were led to an IL Model 181 PICO-metric preamplifier, and displayed on a Tektronix 502A oscilloscope. A Wheatstone bridge circuit with a Devices Isolated stimulator was used to pass current through the recording micro-electrode for direct stimulation, the bridge resistor being 10^9 Ω. As the electrode was advanced into the ganglion, large temporary increases of resistance occurred, presumably due to mechanical obstruction of the tip. Gentle tapping of the micromanipulator baseboard appeared to aid penetration of the outer connective tissue layers of the ganglion. Such tapping produced rhythmic upward (positive) deflexions of the oscilloscope beam, but occasionally these deflexions became reversed in sign. Further advancement of the micro-electrode would then result in the impalement of a ganglion cell, as evidenced by the appearance of a resting potential and responses to stimulation. Cells were frequently impaled without this premonitory sign.

Resting potential. Because the high resistance micro-electrodes used were subject to large changes of tip potential during impalement, measurements of resting potential were very unreliable. Furthermore, in small easily damaged cells the resting potential observed is likely to be as much a measure of the success of impalement as of the true resting membrane potential. Subject to these limitations, the resting potential was usually low (30–50 mV) immediately after impalement, but it often increased over a period of 1–2 min to a value of 50–70 mV.

Observations on most cells were involuntarily terminated by the more or less abrupt disappearance of the resting potential after 2–10 min. Some cells were impaled for as long as 3 hr.

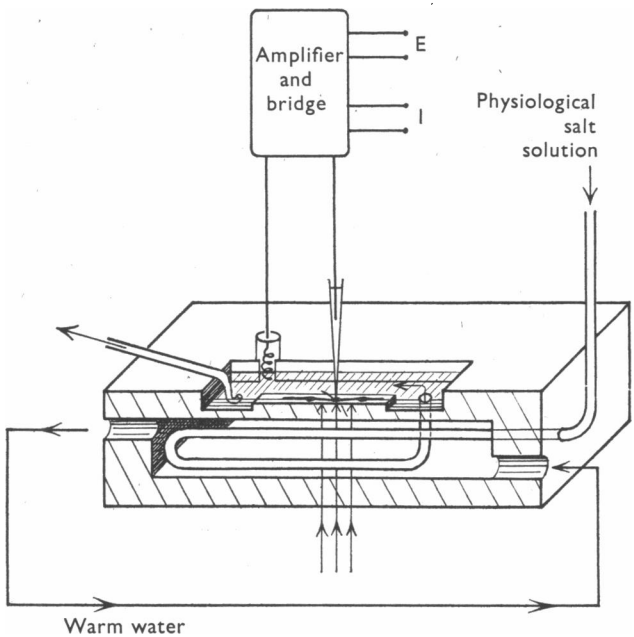

Fig. 1. Cutaway schematic drawing of mounting bath and recording arrangement. The trough and incoming physiological salt solution are warmed by water circulating through the central chamber in the Perspex block. Three separate solution inflow circuits were available (one only is shown). The ganglion preparation straddling the narrow Perspex strip in the middle of the trough is pinned to silicone rubber on either side and transilluminated from below (arrows). The suction electrodes for stimulating the ramus communicans and interganglionic nerves are not shown. Leads are taken from the amplifier and bridge circuit to the oscilloscope vertical amplifiers. Upper beam (E) displays voltage waveforms; lower beam (I) indicates the current during direct stimulation of cells.

RESULTS

Responses to direct stimulation

Electrotonic potentials. Typical responses to the passage of current pulses through the recording micro-electrode are shown in Fig. 2. Hyperpolarizing and subliminal depolarizing pulses gave rise to electrotonic potentials. The

rising and falling phases of these potentials had a mean time constant of 9·1 msec (range 6·1–13·9 msec). The steady-state current–voltage relationship was determined in a number of cells (Fig. 3). Current and voltage were linearly related for all hyperpolarizing currents and usually for depolarization up to 10 mV. The mean input resistance (determined as the slope of the current–voltage curve) was 55·5 MΩ (range 19–186 MΩ). Table 1 shows the input resistance and electrical time constant of eleven cells.

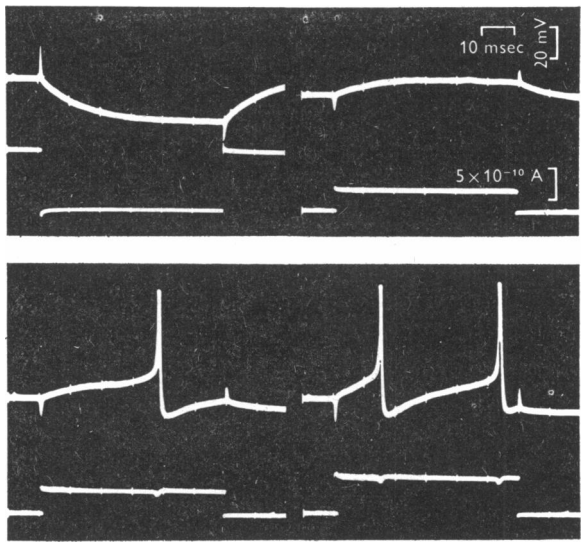

Fig. 2. Electrotonic potentials and action potentials recorded during the passage of current through the micro-electrode. The upper trace of each pair records the intracellular potential with respect to an indifferent bath electrode. The lower trace indicates the amplitude and duration of the current pulse. Cell 20.

TABLE 1. Input resistances and electrical time constants. The input resistance was determined as the slope of the current–voltage curve of each cell. Each time constant shown is the mean of 10–15 individual measurements

Cell number	Input resistance (MΩ)	Time constant (msec)
3	80	7·8
10	25	8·6
11	40	13·9
12	31	6·1
13	30	4·8
14	19	6·7
20	30	11·3
21	43	8·6
24	26	13·5
52	100	7·5
57	186	11·4
	55·5 (±4·8)*	9·11 (±0·91)*

* Mean (±S.E. of mean).

A number of cells gave a 'humped' response to the passage of weak depolarizing current (Fig. 4*a*). In such cases the electrotonic potential decayed into a phase of after-hyperpolarization. The inverse was not observed in response to weak hyperpolarizing current. It is of interest that when synaptic potentials were observed in the same cells, they also showed a phase of after-hyperpolarization (Fig. 4*b*). We were unable to decide between two possible explanations; that the 'hump' represents a transient activation of the Na mechanism responsible for the spike ('local response'), or that it might be similar to the delayed rectification in slow muscle fibres of the frog as observed by Burke & Ginsborg (1956).

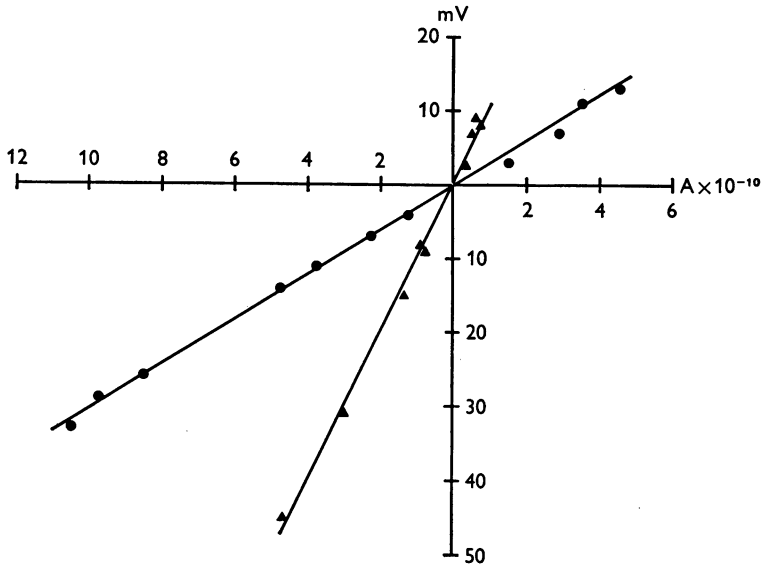

Fig. 3. Steady-state current–voltage relationship for two cells. Cell 13 (circles): R_i = 30 MΩ. Cell 57 (triangles): R_i = 100 MΩ. The first quadrant represents depolarizing responses and the third quadrant represents hyperpolarizing responses.

Action potentials. Ganglion cells discharged action potentials in response to direct stimulation when a critical threshold depolarization of 10–15 mV was reached. The rheobase for long pulses, measured as the current which was just supraliminal, was in the range $1–3 \times 10^{-10}$ A.

The directly evoked action potential showed the following features: (1) a rising electrotonic phase; (2) a spike potential whose peak amplitude was 55–90 mV above the resting base line; (3) a phase of after-hyperpolarization ('after-positivity').

The spike potential was 1·5–2·5 msec in duration, and the after-hyperpolarization recovered by two thirds in 25–30 msec. Ganglion cells fired repetitively in response to strong current pulses (Fig. 4*d*). The maximum

frequency was 80/sec. In most cells a high rate of firing could be maintained almost indefinitely but in a few, the train of spikes was 'damped out' (Fig. 4*d*).

In two cells, depolarizing currents evoked either a subliminal oscillatory response or, with increased current strength, a train of spikes whose minimum frequency was 50/sec (Fig. 4*c*). In these cells it was impossible to evoke a single spike only, with a long (50 msec) current pulse. In the intact animal such cells might conceivably act as high frequency tonic neurones.

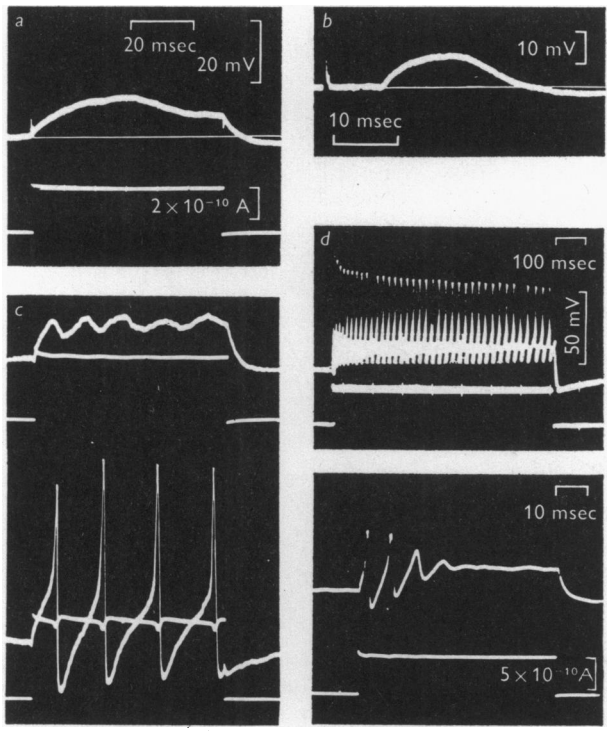

Fig. 4. Further responses to direct stimulation. (*a*) Humped response with after-hyperpolarization. Cell 12. (*b*) Synaptic potential with after-hyperpolarization in a cell (10) with humped direct responses. Stimulation above ganglion. Calibration for (*a*) also applies to (*c*). (*c*) Oscillatory response and (with increased stimulus strength) repetitive firing. Cell 58. (*d*) Upper picture, repetitive firing. Cell 50. Lower picture, damped train of spikes. Cell 49.

However, the observed responses may well have been due to some feature of the *in vitro* conditions; similar behaviour can occur in the squid axon in solutions low in Ca (Huxley, 1959).

'Anode break' spikes, sometimes double, were occasionally observed, particularly in damaged cells whose 'cathodal' spikes were of small amplitude.

Responses to indirect stimulation

General observations. Indirect stimulation evoked either a synaptic potential or an action potential, depending on the stimulus strength. The response occurred after a finite latent period which was roughly proportional to the length of nerve between the stimulating electrode and the ganglion. A stimulus to any of the three inputs gave this response (Fig. 5),

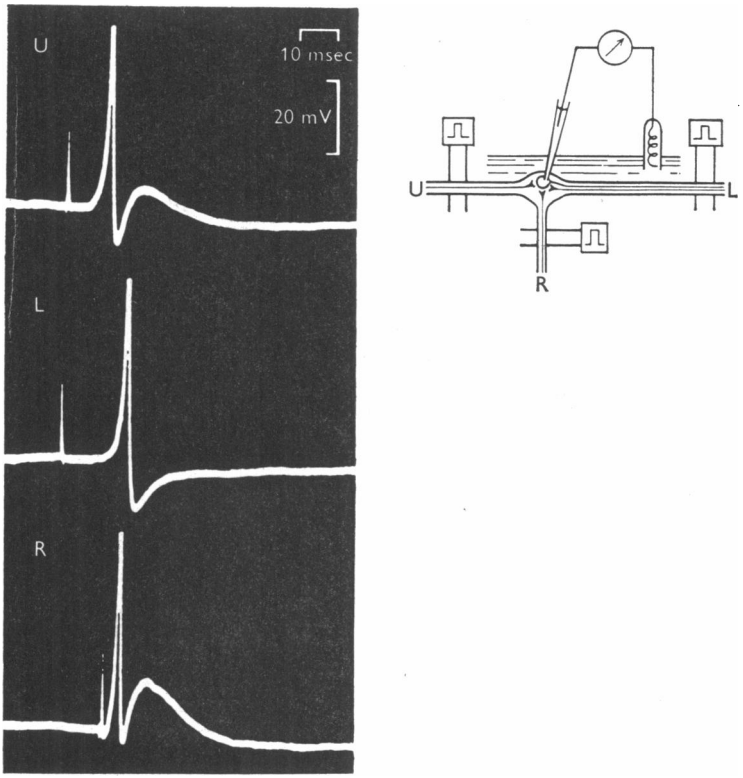

Fig. 5. Response to stimulating each of the three inputs to a ganglion cell. U, interganglionic nerve immediately above ganglion. L, interganglionic nerve immediately below ganglion. R, ramus communicans to ganglion. Note the depolarizing phase following the spike in the U and R responses, indicating greater synaptic activity than in the L response. The latency of the R response is short because of the small length of nerve available.

which was apparently synaptically mediated and due to activation of preganglionic fibres. For each input, synaptic activity could be graded with stimulus strength (Fig. 6*a*). A very weak stimulus evoked no response presumably because it was subliminal for the nerve. At the lowest effective stimulus strength small synaptic potentials were evoked. Increasing the

stimulus strength increased their amplitude. If a synaptic potential reached a critical threshold depolarization level of 10–15 mV an action potential was initiated. As the stimulus strength was still further increased changes in the form of the action potential were apparent. The rate of rise of the synaptic step increased, and the after-hyperpolarization was to some extent counteracted or annulled by a depolarization. In addition the spike amplitude was reduced slightly as a result of the increased intensity of concurrent transmitter activity.

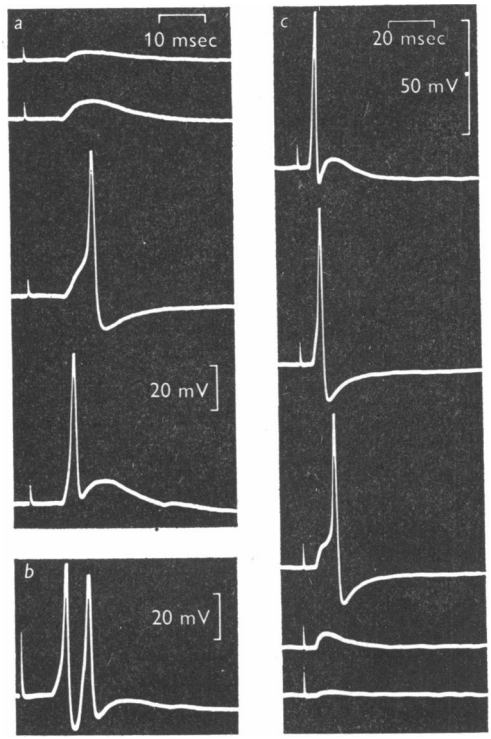

Fig. 6. (*a*) Effect of increasing strength of stimulus to interganglionic nerve above ganglion. Cell 12. From top 8·4, 9·0, 9·5 and 15 V. (*b*) Duration and intensity of synaptic activity sufficient to fire two spikes. Cell 22. Stimulus above ganglion. (*c*) Effect of hexamethonium (10^{-3} M) on the synaptic response. Cell 14. From top, responses to single maximal stimuli above the ganglion before, and 16, 20, 22 and 40 sec after hexamethonium was added to the bath.

In some cells, synaptic activity resulting from a single maximal stimulus to one of the inputs was sufficiently intense and prolonged to fire two or (rarely) three spikes (Fig. 6*b*). This phenomenon has been observed by Erulkar & Woodward (1968) in the superior cervical ganglion of the rabbit, and by Blackman *et al.* (1969) in the pelvic sympathetic ganglia of the guinea-pig. The relevance of this multiple spiking to normal transmission

in the thoracic ganglia is doubtful, since preganglionic activity is unlikely to be in the form of maximal volleys. Nevertheless, the occurrence of double or triple spikes in this way is an indication of the total synaptic activity which can be evoked at any given cell. In the thoracic sympathetic ganglia this activity is likely to be high, since stimulation of one input only (out of three) can give rise to double spikes.

Time course of synaptic potentials. Synaptic potentials had a rising phase with a time to peak of 5–15 msec (mean 9·1 msec) and a slower roughly exponential falling phase whose time constant was 7–18 msec (mean 9·8 msec). Table 2 compares the time constant of decay of synaptic potentials in four cells with the passive electrical time constant of the same cells. Invariably the synaptic time constant is longer.

TABLE 2. Comparison of time constant of decay of synaptic potentials with electrical time constant. Values are shown in each case as the mean of a number (in parentheses) of individual observations. *P* is the probability that the observed difference arose by chance

Cell number	Synaptic potential time constant of decay (msec)	Electrical time constant (msec)	*P*
3	12·8 (16)	7·8 (6)	< 0·001
12	8·7 (14)	6·1 (21)	< 0·001
14	9·3 (12)	6·7 (13)	< 0·001
52	15·9 (4)	7·5 (9)	< 0·001

Block by tubocurarine and hexamethonium. Tubocurarine (5×10^{-4} M) in one experiment reduced the synaptic activity following a single maximal volley to a level which was just subliminal for the spike, although over 5 min was required before block occurred after changing over to the drug-containing solution.

Hexamethonium was a more rapidly acting blocking agent. Block occurred 15–30 sec after changing to the drug solution (10^{-3} or 5×10^{-4} M). Figure 6*c* shows the effect of 10^{-3} M hexamethonium. Synaptic activity is eventually reduced to a very low level.

Antidromic responses. Unequivocal evidence of antidromic stimulation was seldom obtained. In two cells (17 and 70), an all-or-none response to stimulation of the lower end of the sympathetic chain was observed (Fig. 7*a*, *b*). In both cells the latency of the all-or-none response was about twice that of the synaptic response, but the threshold was higher for cell 17 (Fig. 7*a*) and lower for cell 70 (Fig. 7*b*).

The all-or-none response illustrated in Fig. 7*a* could not have been antidromically conducted: it would have been annulled by collision with the synaptically generated action potential travelling down the axon. It is possible, however, that the all-or-none response illustrated in the upper part of Fig. 7*b* is antidromic.

All-or-none responses were unmasked in three cells treated with hexamethonium. Evidently these responses were antidromically conducted. In one cell (Fig. 7*c*), the unmasked antidromic action potential showed a step and plateau on its rising phase. After several minutes the spike failed to take off from this plateau.

In one experiment, the superior cervical ganglion was used in place of a thoracic ganglion. Stimulating electrodes were placed on the pre- and post-ganglionic trunks. Antidromic stimulation again evoked a response (Fig. 7*d*) with a step and plateau as in cell 12. The step and plateau apparently represent a region of reduced safety factor of propagation of the antidromic spike into the soma of the ganglion cell. The axon spike would then be recorded electrotonically.

Fibre types and latency of response. The response to indirect stimulation occurred after a finite latent period which was measured for each of the

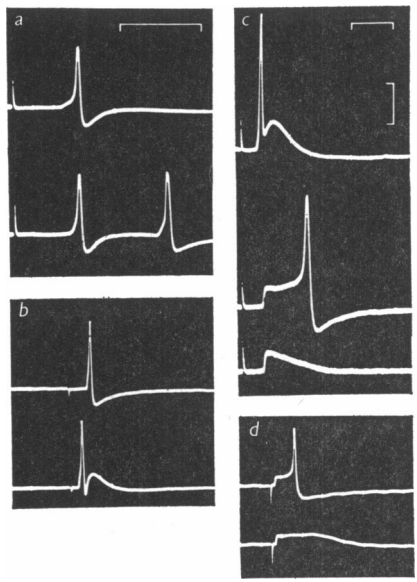

Fig. 7. All-or-none responses produced, by stimulation of the sympathetic chain below the ganglion (in *a*, *b*, *c*) or of the post-ganglionic nerve trunk (in *d*). (*a*) Upper trace, orthodromic action potential alone; lower trace, with increased stimulus strength an all-or-none orthodromic action potential follows the first response. See text for explanation. Cell 17. (*b*) Upper trace, antidromic response; lower trace, increased stimulus strength evokes orthodromic response of shorter latency. Cell 70. (*c*) Upper trace, response to a single maximal stimulus in the absence of hexamethonium; middle trace, all-or-none response remaining after orthodromic response blocked with hexamethonium (10^{-3} M); lower trace, antidromic spike fails to invade soma. Cell 12. (*d*) Superior cervical ganglion. Note failure to invade in lower trace. Cell 36. Vertical calibration: 40 mV in (*a*), (*b*), (*d*), 20 mV in (*c*). Horizontal calibrations; 30 msec in (*a*) and (*c*). The calibration for (*c*) also applies to (*b*) and (*d*).

three inputs as the time between the stimulus artifact and the beginning of the synaptic potential. The distance from the stimulating electrodes to the ganglion was also estimated. The quotient of these is the apparent conduction velocity of the fastest fibres innervating the cell. The true conduction velocity would be somewhat greater than this value since a part of the latent period is occupied by the 'synaptic delay'. An attempt was made to estimate the synaptic delay in the following way. The latency of the synaptic response in each cell was plotted against the corresponding nerve length. The synaptic delay should appear as an intercept of the regression line on the latency axis. Unfortunately the scatter of the results made an accurate estimate impossible.

In a small number of cells, a single stimulus evoked two or sometimes three synaptic potentials of different latency and threshold. This might indicate innervation of these cells by groups of fibres of different conduction velocities.

A wide range of apparent conduction velocities was found (0·17–1·1 m/sec). A histogram (Fig. 8) showed the distribution to be unimodal, and two populations could not be distinguished by eye.

To discover the fibre population of the sympathetic chain external, recordings from a short ganglion-free length of the chain were made in two experiments. Figure 9 shows the train of potentials evoked by a single maximal stimulus. There are two main deflexions, corresponding to conduction velocities of 5–15 m/sec and 0·2–1·0 m/sec. The second deflexion had a higher threshold than the first and could not readily be made monophasic by crushing the nerve between the recording electrodes. It was probably, on this account, compounded chiefly of a large number of C fibre responses, whereas the fast responses were those of comparatively few large B fibres.

Spontaneous synaptic potentials. Small spontaneously occurring depolarizations resembling in form evoked synaptic potentials were observed in most cells. Their occurrence frequency was low but increased during indirect stimulation (Fig. 10*a*). The time to peak of these 'miniature' synaptic potentials was shorter than that of evoked synaptic potentials. For example, in cell 3 the mean time to peak of spontaneous potentials was $4{\cdot}0 \pm 0{\cdot}3$ (S.E. of mean) msec, whereas the mean time to peak of evoked potentials was $6{\cdot}8 \pm 0{\cdot}3$ (S.E. of mean). This difference was highly significant ($P < 0{\cdot}001$). The mean amplitude of spontaneous potentials in five cells is given in Table 3. The amplitude distribution for three cells is shown in Fig. 10*b* and Fig. 13. The distribution is positively skewed as at the frog sympathetic ganglion (Blackman, Ginsborg & Ray, 1963*b*) and chick ciliary ganglion (Martin & Pilar, 1964). There was no inverse correlation between amplitude and time to peak of spontaneous potentials.

It is unlikely, therefore, that the skewing arises because quanta are released at different distances from the recording electrode, for example, along dendrites. Skewing might be due to the occurrence of multiquantal discharges resulting from brief non-random interactions between spontaneous events as discussed by Martin (1966); but, of course, it is possible that the quanta of release themselves have a skewed distribution.

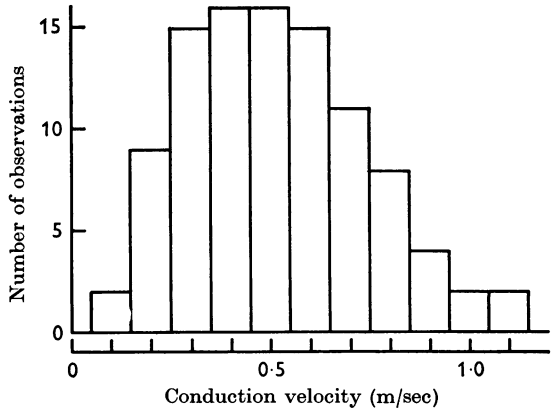

Fig. 8. Histogram of conduction velocities of preganglionic fibres as determined by latency measurements.

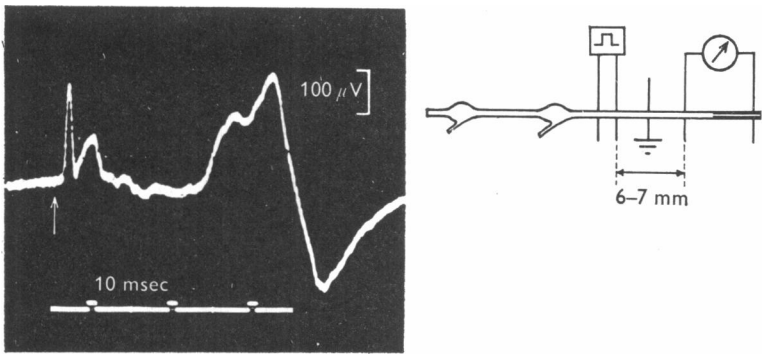

Fig. 9. External recording from the interganglionic nerve. The distance between the stimulating cathode and the 'active' recording electrode was 6–7 mm. The photograph shows the response to a single maximal stimulus. The position of the stimulus artifact is indicated by an arrow. Time marker, 10 msec per division.

If the resting potential E_m and transmitter reversal potential E_{ACh} are known, it is possible to calculate the mean number of quanta making up a synaptic potential of any given size. If n is the number of quanta

$$n = \frac{E_{sp}(E_m - E_{ACh} + E_{spon})}{E_{spon}(E_m - E_{ACh} + E \quad)},$$

where E_{sp} is the synaptic potential amplitude and E_{spon} is the mean amplitude of spontaneous potentials. Although E_m is not known accurately, and E_{ACh} is not known at all, reasonable values are $E_m = -55$ mV, and $E_{ACh} = -15$ mV as at other sites. Putting $E_{sp} = 15$ mV (i.e. approximately threshold depolarization) and inserting values of E_{spon} from Table 3, $n = 8-20$. This then is a rough estimate of the number of quanta which when released simultaneously will cause the discharge of an action potential.

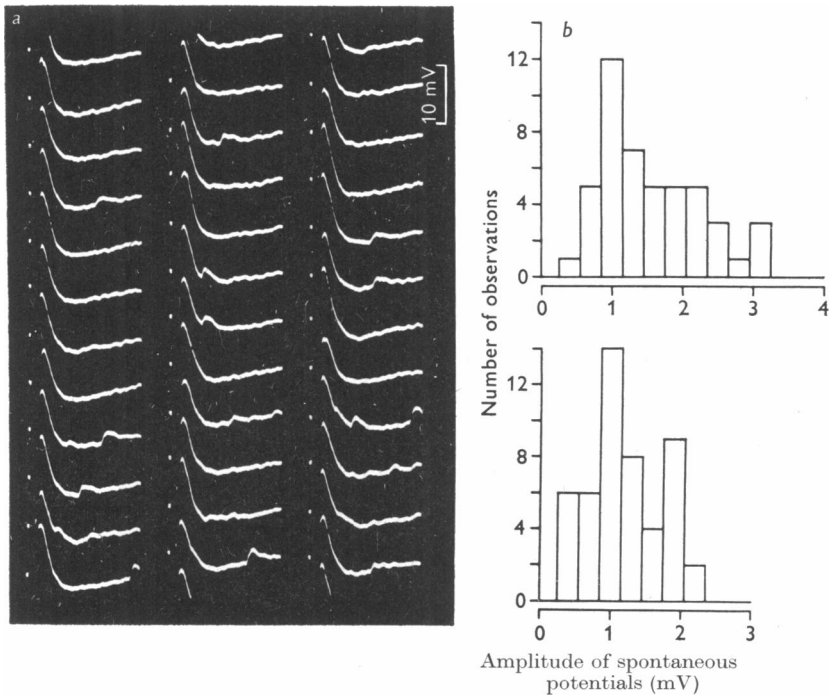

Fig. 10. (*a*) Spontaneous synaptic potentials. The occurrence frequency has been increased by indirect stimulation above the ganglion at a frequency of 2/sec. The spikes have been obliterated during photographic processing, but the after-depolarizations and after-hyperpolarizations can be seen. (*b*) Amplitude distributions of spontaneous synaptic potentials in two cells. Upper histogram, cell 12. Lower histogram, cell 57.

TABLE 3. Amplitude of spontaneous potentials. Values are expressed as mV ± S.D. with the number of observations in parentheses

Cell number	Mean amplitude
3	3·0 ± 0·7 (5)
12	1·5 ± 0·7 (47)
57	1·2 ± 0·5 (49)
80	1·9 ± 0·6 (83)
82	1·5 ± 0·4 (8)

High frequency indirect stimulation markedly increased the occurrence frequency of spontaneous potentials. Following such stimulation, the frequency declined exponentially with a time constant of about 20 sec (Fig. 11).

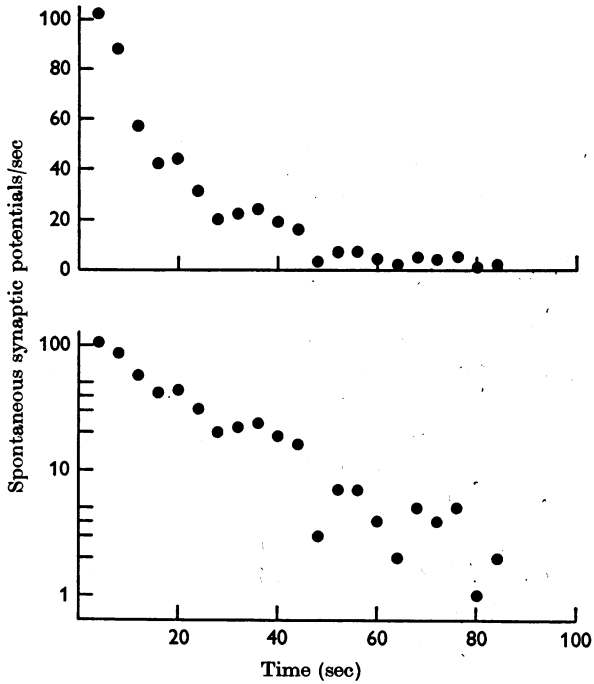

Fig. 11. Occurrence frequency of spontaneous synaptic potentials following a conditioning tetanus (maximal stimuli, 30/sec for 20 sec) to the interganglionic nerve above the ganglion. Lower graph is a semi-logarithmic plot of the same results. Cell 80.

Grading and quantum content. Grading of synaptic potentials with stimulus strength is a function of the number of preganglionic fibres innervating the ganglion cell. Recruitment of fibres with increasing stimulus strength should give rise to a stepped relationship between stimulus strength and response amplitude. However, considerable fluctuation in the amplitude of successive synaptic potentials in response to indirect stimuli of constant strength (Fig. 12) obscured this relationship. In order to overcome this difficulty and to estimate the number of fibres recruited up to firing threshold, experiments were performed in which the stimulus strength was increased by small increments. Responses to between twenty and fifty stimuli were recorded at each level. A period of 1 min of stimulation was allowed at each new level before recording was started.

In cell 82, roughly half the responses at the lowest effective stimulus strength were 'failures'. The responses which did occur had a mean amplitude of 2·2 mV (Table 4). At the next three higher stimulus levels this mean response amplitude was not significantly altered. There were, however, no failures. Evidently the lowest stimulus strength was threshold for only one nerve fibre; at the next three levels no new fibres were stimulated. As shown in Table 4, further increase in stimulus strength resulted in a second mean level of response being attained, presumably by the stimulation of a second fibre. At the highest stimulus strength shown, a third

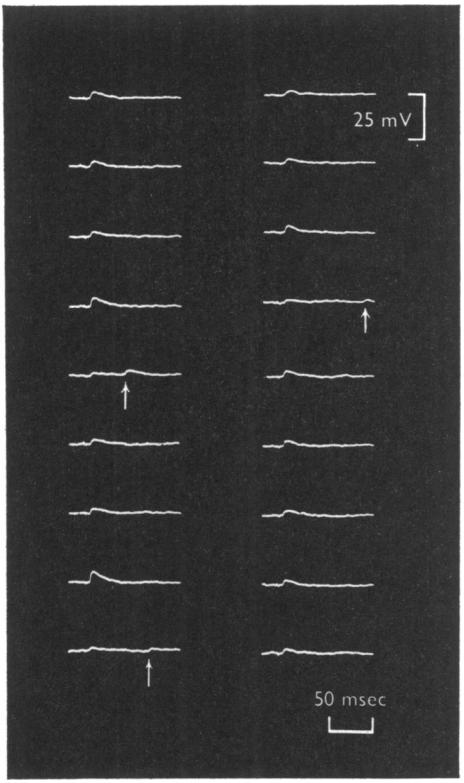

Fig. 12. Fluctuation of synaptic potentials at constant stimulus strength. Cell 82. Stimulus above ganglion at a frequency of 1/sec. Arrows indicate spontaneous synaptic potentials.

level of response was reached. A proportion of these responses gave rise to action potentials in the ganglion cell. At this level, three or more fibres must have been active.

A number of spontaneous synaptic potentials were recorded during this experiment. Assuming these to represent unit packets of transmitter

release, we have calculated the mean quantum content of responses at each stimulus level (Table 4). The mean quantum content of synaptic potentials occurring at the four lowest stimulus levels was 1·5–1·8. This, then, is a measure of the number of quanta released by an impulse in a single preganglionic nerve fibre supplying a ganglion cell.

TABLE 4. Grading and quantum content of synaptic potentials

Cell number	Stimulus strength (V)	Number of stimuli	Number of zero responses	Mean response amplitude (mV)	Mean amplitude of spontaneous potentials (mV)	Mean quantum content
82	3·6	21	12	2·2*		1·5
	4·0	20	0	2·4		1·6
	4·5	20	0	2·6		1·7
	5·0	20	0	2·4	1·5	1·6
	5·5	20	0	4·2		2·8
	6·0	20	0	3·8		2·5
	6·5	20	0	8·0†		—
80	6·0	44	18	0·8‡		0·4
	6·2	50	12	1·4‡		0·7
	6·4	50	2	2·6‡	1·9	1·4
	6·6	52	1	2·8‡		1·5
	6·8	57	1	2·7‡		1·4

* Mean amplitude of synaptic potentials ignoring zero responses.

† Mean of six values; the other fourteen responses gave rise to action potentials.

‡ Mean includes zero responses.

It is possible that this estimate is too low. We may have overestimated the mean size of the quantum of release. This would occur if the skewing of amplitude distribution of spontaneous potentials was due to a proportion of multiquantal discharges. In view of the small mean number of quanta released by an impulse, it is remarkable that suprathreshold stimuli to the fibre always gave a synaptic response. Apparently, the probability of release of quanta was high, sufficiently high to ensure the release of at least one quantum by each impulse. If the low mean quantum content of synaptic potentials was due to a low probability of release, the number of quanta comprising successive responses could be expected to conform to a Poisson distribution, in which case about 20% of stimuli should have released no quanta (cf. Blackman *et al.* 1963*c*).

In another experiment (cell 80), similar conclusions were reached. In this cell, the mean number of quanta released from a single fibre by an impulse must have been even smaller than in cell 82. As can be seen from Table 4, it is not possible to conclude that at the two lowest stimulus levels only one nerve fibre was consistently being stimulated. However, at the three highest stimulus levels, the mean response amplitude was constant; evidently the same number of nerve fibres (at least two) were being stimulated. During the experiment, eighty-three spontaneous synaptic potentials

were recorded (see amplitude distribution in Fig. 13*a*). This allowed an estimate to be made of the mean quantum content (1·4–1·5) of synaptic potentials at this response level. If a Poisson distribution is assumed for the number of quanta released by each stimulus, the proportion of zero responses provides an independent estimate of the mean (3·7). The unit size (0·73 mV) derived from this value is, however, smaller than almost all the recorded spontaneous potentials. Figure 13 compares the amplitude distribution of the pooled responses at the upper level (*b*) with that of the concurrently recorded spontaneous potentials (*a*).

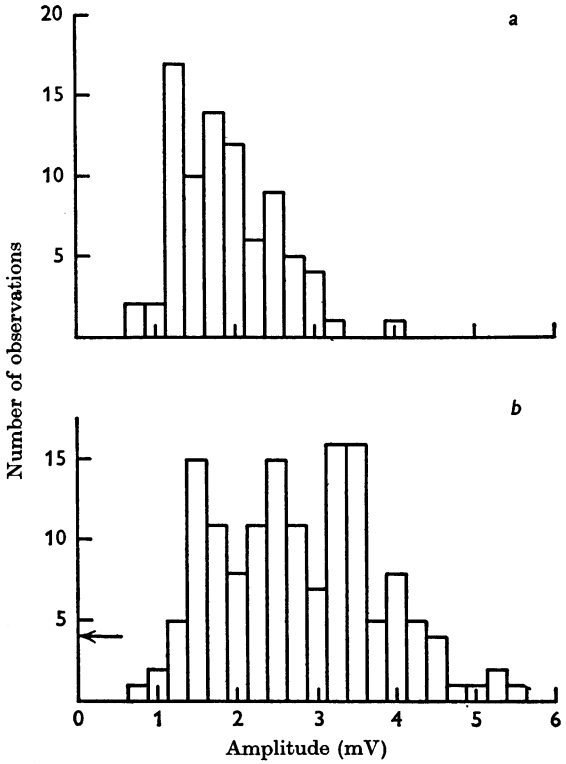

Fig. 13. Amplitude distribution of (*a*) spontaneous synaptic potentials and (*b*) synaptic potentials evoked by stimulation above the ganglion at a frequency of 1/sec. The arrow indicates the number of zero responses or 'failures'. Cell 80.

Facilitation. Following a period of rest, the amplitude of synaptic potentials in response to successive submaximal indirect stimuli was not constant but increased over a period to reach a higher value. Figure 14 shows the time course of the increase in synaptic potential which occurred in cell 80 following the commencement of stimulation at a frequency of 2/sec. The facilitated synaptic potentials occasionally reached spike thres-

hold for the ganglion cell as indicated by the arrows in the graph. A semi-log. plot (not shown) suggested that the facilitation followed an exponential time course.

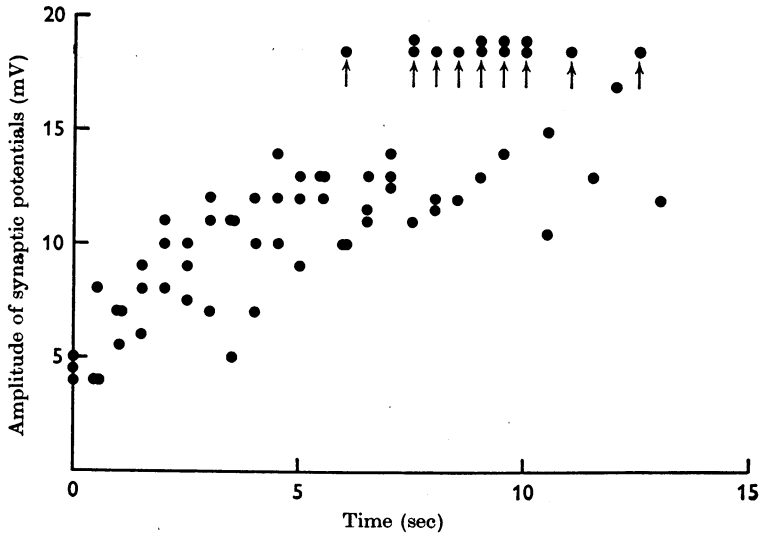

Fig. 14. Facilitation of synaptic potentials following commencement of stimulation. The graph combines the results of three runs. Stimulus frequency in each run was 2/sec. Arrows indicate the generation of a spike. Cell 80.

Post-tetanic potentiation. Following a conditioning tetanus, the mean synaptic response to submaximal test stimulation of the same input was usually increased in amplitude for a few minutes (homonymous post-tetanic potentiation), although in some cells this potentiation was preceded by an initial phase of depression lasting about 10 sec. In Fig. 15 (*a* and *b*), the tetanic and test stimuli were applied to the sympathetic chain above the ganglion. An unusual feature illustrated in Fig. 15*a* was the reduced fluctuation in amplitude of synaptic potentials during the first 50 sec following the tetanus.

After-discharges and slow responses. One cell (74), which was otherwise normally responsive, gave rise to an after-discharge (Fig. 16) following repetitive stimulation of the sympathetic chain above the ganglion (0·75–2·0 sec, 30–80 c/s). For the first 0·1–0·2 sec of stimulation the cell fired at the stimulus frequency. There then followed a period during which the frequency fell off (the cell failed to respond to some stimuli) but about 1 sec from the start, the firing frequency increased again. An after-discharge followed the end of stimulation. Following a short train (Fig. 16*a*) the after-discharge began about 1 sec after the start of stimulation: this

corresponds to the time at which the firing frequency increased again during long trains (Fig. 16*b*, *c*). Some excitatory process which took roughly 1 sec to reach threshold was apparently initiated at the onset of

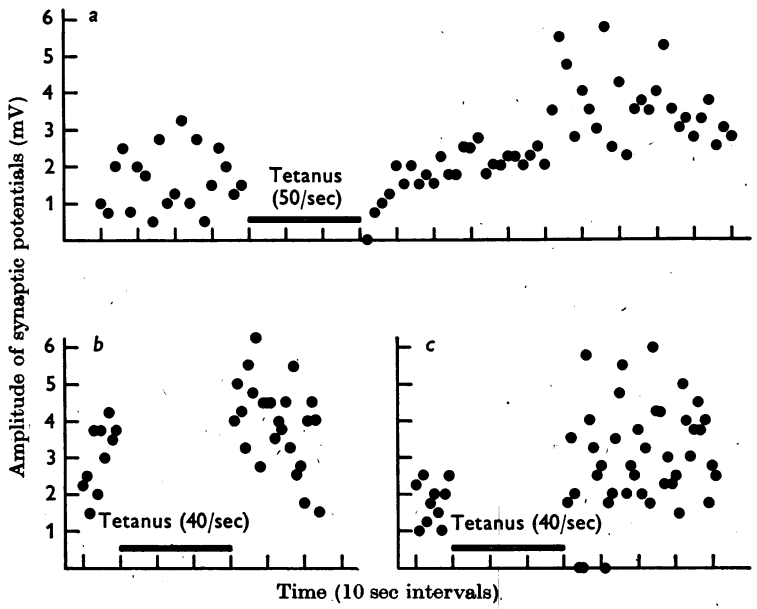

Fig. 15. Post-tetanic potentiation. (*a*) Effect of homonymous tetanus on response to submaximal stimulation (frequency 0·5/sec) above ganglion. Horizontal bar indicates a train of maximal stimuli (50/sec for 32 sec) Cell 80. (*b*) As in (*a*) except that stimulus frequency was 1/sec, and the tetanus was 40/sec for 30 sec. Cell 82. (*c*) Effect of heteronymous tetanus on response to submaximal stimulation (frequency 1/sec) above ganglion. Horizontal bar indicates a train of maximal stimuli (40/sec for 30 sec) to the interganglionic nerve below ganglion. Cell 82.

stimulation in each case. The maximum frequency of firing during the after-discharge was 15/sec. It may be noted here that this cell had earlier during the experiment been exposed to a concentration of tubocurarine sufficient to block the spike response to maximal indirect stimulation. The tubocurarine was subsequently washed out with apparently complete recovery of the synaptic response. Only a few experiments were done which would have allowed the observation of after-discharge or other slow synaptic responses corresponding to those reported to occur in repetitively stimulated amphibian sympathetic ganglia (Tosaka, Chichibu & Libet, 1968; Kobayashi & Libet, 1968; Nishi & Koketsu, 1968). With the above exception, none was observed.

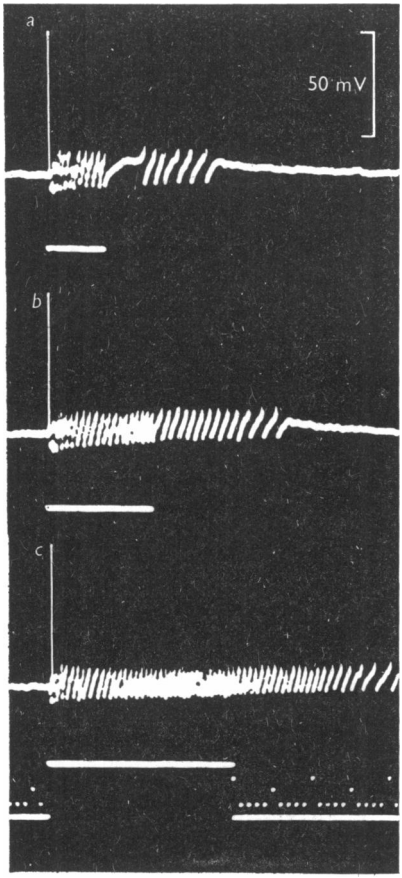

Fig. 16

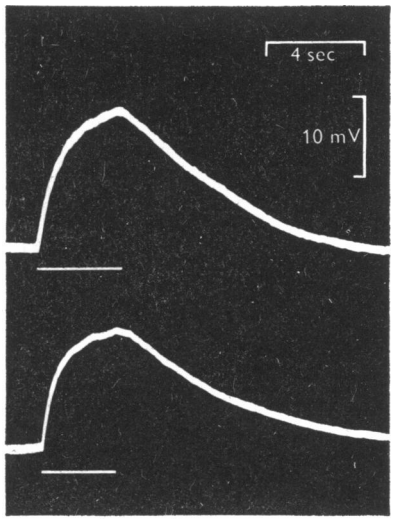

Fig. 17

Fig. 16. After-discharge following repetitive maximal stimulation of the interganglionic nerve above the ganglion. Stimulus frequency 40/sec in (*a*) and (*b*), and 60/sec in (*c*). Stimulus time shown by horizontal bars. Time marker, 100 msec. Faint recorded spikes not shown; first spike of each train drawn in. Cell 74.

Fig. 17. Slow depolarizations recorded from an inexcitable cell during repetitive stimulation (40/sec) of interganglionic nerve below ganglion (upper trace) and above (lower trace). Horizontal bars indicate time during which stimulation was carried out. Cell 43.

Inexcitable cells

Cells of a second type were frequently impaled. These cells were characterized by a high resting potential, a very low input resistance, electrical inexcitability and lack of response to single indirect stimuli. Repetitive indirect stimulation usually gave rise to a slow depolarization (Fig. 17). This could be as large as 20 mV, and decayed with a time constant of

5–7 sec. The depolarizing response was graded with stimulus frequency and strength.

Fibre pathways

Stimulation of the ramus communicans or of the sympathetic chain immediately above or below a ganglion invariably gave a graded synaptic response in impaled ganglion cells. It may be concluded that many preganglionic fibres in connexion with any given ganglion cell run in these nerve trunks. To determine how far preganglionic fibres ascend or descend in the chain, a longer length was dissected out in some experiments so that one or two ganglia intervened between the stimulating electrodes and the site of recording. The stimulating arrangements are summarized in Fig. 18,

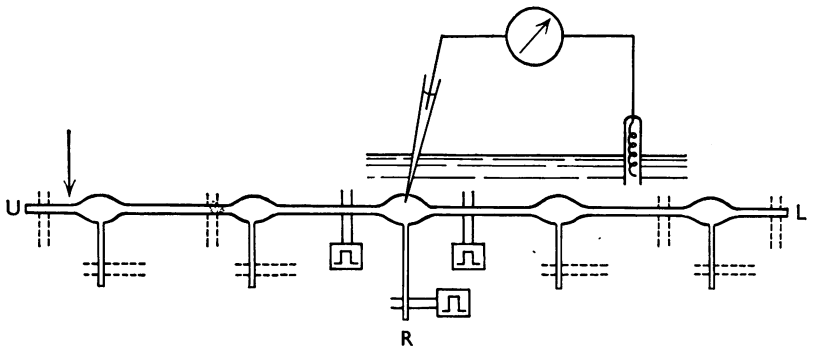

Fig. 18. Schematic diagram showing the places in the sympathetic chain at which stimulation in different experiments could evoke synaptic responses in impaled cells. In one experiment, stimulation at the place indicated by the arrow failed to give a response in any of the cells impaled. U, upper end of sympathetic chain; L, lower end of sympathetic chain; R, ramus communicans.

which combines the results of all the experiments. Stimulation at any of the places indicated gave a graded response in impaled cells. In one experiment stimulation three segments above the site of recording (as shown by arrow) did not give a response in any of the cells impaled. Preganglionic fibres can apparently ascend at least three segments, or descend two. It may be that at this level they do not descend more than two segments.

The question arises whether preganglionic fibres make synapses in ganglia other than those in which they terminate. The two arrangements possible in a ganglion are illustrated in Fig. 19. Since preganglionic fibres can ascend or descend in the chain, it is possible that they make synapses *en passant* (Fig. 19*a*). Obviously fibres must terminate *somewhere*, so that at least some component of the innervation of the cell will be as in Fig. 19*b*.

We have considered several methods for determining whether the *en*

passant arrangement occurs. In preliminary experiments, a method employing the phenomenon of post-tetanic potentiation was applied in an attempt to decide whether the *en passant* arrangement occurs.

If such an arrangement is present, a tetanus applied to one input to the ganglion should potentiate the response to a submaximal test stimulus to another input, since some of the same synapses would be activated by both the tetanus and the test stimulus. We were able to carry out such an experiment successfully in only one cell. The result is shown in Fig. 15*c*. The test responses were markedly potentiated following the tetanus.

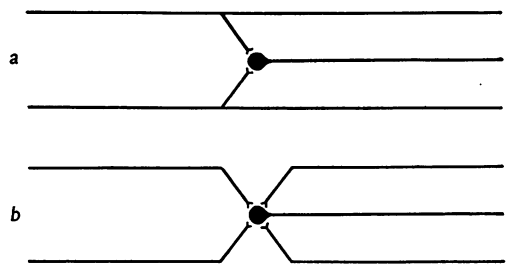

Fig. 19. Innervation of ganglion cell by preganglionic fibres. (*a*) *En passant* arrangement; (*b*) 'normal' arrangement.

DISCUSSION

The electrical properties of these cells were (mean values): input resistance 55 MΩ; electric time constant 9·1 msec; input capacitance 0·17 nF. A preliminary histologic study kindly carried out by Mr C. E. Devine using both light and electron microscopy has shown that the cells are prolate spheroids with mean major and minor diameters of 31 and 19 μ respectively. The calculated surface area is $1{\cdot}8 \times 10^{-9}$ m^2, giving a resistance of unit area of membrane $R_m = 1000\ \Omega$ cm^2 and a capacitance of unit area of membrane $C_m = 10\ \mu$F/cm^2.

Although the cells are known to have dendrites, no allowance has been made for them in the calculation of membrane area. The value of R_m is therefore too small by an unknown factor, and the value of C_m is too large by the same factor. The values obtained are comparable to those obtained in pelvic sympathetic ganglion cells of the guinea-pig (Blackman *et al.* 1969) and within one order of magnitude of those found at other sites (Katz, 1966).

Cells with similar membranes should have the same time constant independent of cell size. The time constants shown in Table 1 cover a wide range. Three reasons for this variation present themselves: differences in electrical leakage around the microelectrode tip; anisopotential conditions within the cell; actual differences of membrane characteristics. The extent

to which these could affect the measured time constant is not known, but some of the experiments indicated that the measured input resistance could either increase or decrease by as much as 50% over a period of 20 min. This would be most easily explained by variations in the perfection of the seal around the tip of the micro-electrode. Since these cells have dendrites, isopotential conditions certainly do not prevail internally. Differences of anatomical structure of cells are therefore likely to lead to different time constants. Finally, there is the possibility that real differences of R_m and C_m occur. In frog sympathetic ganglia there appear to be two types of ganglion cell, named by Nishi, Soeda & Koketsu (1965) sB and sC. These two types are innervated by, and give rise to, B fibres and C fibres respectively, and their membrane characteristics differ slightly. It has not been possible to distinguish two types in the guinea-pig sympathetic ganglia, although this cannot, of course, be interpreted as evidence that these two types are not in fact present.

Table 2 shows that the time constant of decay of synaptic potentials is greater than the passive electric time constant. A sufficient explanation would be that weak synaptic activity continues during the falling phase, as has been postulated for other ganglionic sites (Nishi & Koketsu, 1960; Blackman *et al.* 1963*a*).

The time to peak of evoked synaptic potentials was longer than that of spontaneous synaptic potentials (p. 183). This is likely to be due to asynchrony of arrival of preganglionic impulses. Small differences of conduction velocity or fibre-path length could account for the temporal dispersion.

The fibre spectrum of the sympathetic chain as determined by external recording of the compound nerve action potential consisted of a large number of slowly conducting fibres with a smaller group of relatively fast fibres. The electron microscopic studies (C. E. Devine, personal communication) have provided the following information: (1) both myelinated and non-myelinated fibres are found in the sympathetic chain, in the approximate ratio of 1:8, (2) the diameter of the non-myelinated fibres was 0·5–1·0 μ, (3) the diameter of most of the myelinated fibres was 0·5–1·5 μ, but some were larger (3–5 μ). It is known that myelinated and non-myelinated fibres conduct at the same velocity (about 1 m/sec) when their diameters are 1–2 μ (Rushton, 1951). It is therefore proposed that the large slow group of fibres was composed of both myelinated and non-myelinated fibres. Evidently B and C fibres could not be distinguished purely on grounds of conduction velocity. The intracellular recording experiments have not so far shown responses of short latency corresponding to the fast fibres described above. All of the responses (both orthodromic and antidromic) were of long latency, conducted by fibres whose conduction velocity would place them in the slow group. Nishi *et al.* (1965)

in amphibian lumbar sympathetic ganglia distinguished B and C fibres from latency measurements. They obtained a bimodal distribution of conduction velocities. Both B and C fibres may innervate mammalian sympathetic ganglion cells, but our results do not allow the distinction. The function of the fast fibres is open to conjecture. They may be related to ganglion cells which are, for some reason, inaccessible to microelectrode penetration. Alternatively, they may be sensory, their cell bodies lying in the dorsal-root ganglia. Such fibres would pass through sympathetic ganglia without making synapses.

It is noteworthy that impulses in single preganglionic fibres to a ganglion cell appear to release on average only about one quantum of transmitter. Since the release at this low level did not conform to a Poisson distribution (the proportion of zero responses was considerably lower than would be expected) we have concluded that the population of quanta available for release at any moment is small, but that the probability of release is relatively high.

In amphibian lumbar sympathetic ganglia, estimates of the mean number of quanta released by a single nerve impulse range from 25 to 130 (Blackman *et al.* 1963*c*; Nishi *et al.* 1967). Nishi *et al.* (1967) calculated that a synaptic knob released about 2–5 quanta with each impulse. In the mammalian preparation, the mean number, assuming that a preganglionic fibre has only one synaptic knob on each ganglion cell, appears to be of the order of one quantum. If, as is likely, there are several knobs, the mean quantal release per knob would be very low indeed. On the limited basis of our experiments, it would appear that rather more than three fibres must be simultaneously active for transmission to occur. The total number of fibres innervating each ganglion cell is not known. An estimate might be made by comparing the initial rate of spontaneous potentials with that of the response to a maximal simultaneous volley from all three inputs. Provided the quantum content of a single fibre response is known, the number of active fibres may be calculated. A source of inaccuracy, however, would be the asynchrony of release of quanta.

We have concluded that preganglionic fibres in the thoracic sympathetic chain may either ascend or descend several segments after emerging from the spinal canal, and that each fibre probably makes synapses in more than one ganglion. Langley (1899), from observations of piloerection in cats following stimulation of various parts of the sympathetic nervous system, concluded that preganglionic fibres in the thoracic region ascended four segments, making synapses in each ganglion. It is possible that the discrepancy arises from the use of ganglia at different levels; in the lumbar region all preganglionic fibres descend (Langley, 1899). The level at which we recorded responses was presumably transitional between a purely

descending (lumbar) and a purely ascending (cervical and upper thoracic) pattern. Obviously, a systematic study of this problem using both extracellular and intracellular recording methods is needed.

The nature of the inexcitable cells is not known. They are similar to those impaled by Blackman *et al.* (1969) in pelvic sympathetic ganglia. It is possible that they are simply artifacts of recording associated with penetration of ganglion tissue. However, it has been shown (Blackman *et al.* 1969) that increase of K causes depolarization; this was interpreted as evidence of their cellular nature, possibly glial or chromaffin cells. In the thoracic cells, the slow depolarization in response to repetitive indirect stimulation may have been synaptically mediated, the result perhaps, of a slow build-up and diffusion of acetylcholine. Alternatively, it may have been caused by a rise in the extracellular concentration of K consequent on its release from neighbouring sympathetic fibres and neurones. Studies of the pharmacology of the slow depolarization would be of interest; they might shed light on the physiology. After-discharges following repetitive stimulation have been recorded intracellularly in amphibian nicotinized or curarized sympathetic ganglia by Nishi & Koketsu (1968) and Tosaka *et al.* (1968). We did not systematically search for this phenomenon in our preparation. An after-discharge was, however, seen in one normally transmitting cell. The time course of the depolarization causing the after-discharge suggests that it corresponds to the extracellularly recorded LN wave described by Eccles (1952). The depolarization appears to be the result of the late action of acetylcholine delayed by diffusion to a site remote from its place of release (Eccles & Libet, 1961).

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REFERENCES

BLACKMAN, J. G., CROWCROFT, P. J., DEVINE, C. E., HOLMAN, MOLLIE, E. & YONEMURA, K. (1969). Transmission from preganglionic fibres in the hypogastric nerve to peripheral ganglia of male guinea-pigs. *J. Physiol.* **201**, 723–743.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*a*). Synaptic transmission in the sympathetic ganglion of the frog. *J. Physiol.* **167**, 355–373.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*b*). Spontaneous synaptic activity in sympathetic ganglion cells of the frog. *J. Physiol.* **167**, 389–401.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*c*). On the quantal release of the transmitter at a sympathetic synapse. *J. Physiol.* **167**, 402–415.

BLACKMAN, J. G. & HOLMAN, M. E. (1967). Intracellular recordings from mammalian sympathetic ganglion cells. *Proc. Univ. Otago med. Sch.* **45**, 52–53.

BOYD, J. D. (1957). Intermediate sympathetic ganglia. *Br. med. Bull.* **13**, 207–212.

BURKE, W. & GINSBORG, B. L. (1956). The electrical properties of the slow muscle fibre membrane. *J. Physiol.* **132**, 586–598.

ECCLES, R. M. (1952). Responses of isolated curarized sympathetic ganglia. *J. Physiol.* **117**, 196–217.

ECCLES, R. M. (1955). Intracellular potentials recorded from a mammalian sympathetic ganglion. *J. Physiol.* **130**, 572–584.

ECCLES, R. M. (1963). Orthodromic activation of single ganglion cells. *J. Physiol.* **165**, 387–391.

ECCLES, R. M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol.* **157**, 484–503.

ERULKAR, S. D. & WOODWARD, J. K. (1967). Transmembrane potentials recorded from neurones of rabbit superior cervical ganglion. *J. Physiol.* **192**, 45-46*P*.

ERULKAR, S. D. & WOODWARD, J. K. (1968). Intracellular recording from mammalian superior cervical ganglion *in situ*. *J. Physiol.* **199**, 189–203.

FOLEY, J. C. & SCHNITZLEIN, H. N. (1957). The contribution of individual thoracic spinal nerves to the upper cervical sympathetic trunk. *J. comp. Neurol.* **108**, 109–120.

HUXLEY, A. F. (1959). Ion movements during nerve activity. *Ann. N.Y. Acad. Sci.* **81**, 221–245.

KATZ, B. (1966). In *Nerve, Muscle and Synapse*. New York: McGraw-Hill.

KOBAYASHI, H. & LIBET, B. (1968). Generation of slow postsynaptic potentials without increases in ionic conductance. *Proc. natn. Acad. Sci. U.S.A.* **60**, 1304–1311.

LANGLEY, J. N. (1899). On axon-reflexes in the pre-ganglionic fibres of the sympathetic system. *J. Physiol.* **25**, 364–398.

LIBET, B. & TOSAKA, T. (1966). Slow postsynaptic potentials recorded intracellularly in sympathetic ganglia. *Fedn Proc.* **25**, 270.

MARTIN, A. R. (1966). Quantal nature of synaptic transmission *Physiol. Rev.* **46**, 51–66.

MARTIN, A. R. & PILAR, G. (1964). Quantal components of the synaptic potential in the ciliary ganglion of the chick. *J. Physiol.* **175**, 1–16.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.

NISHI, S. & KOKETSU, K. (1968). Early and late afterdischarges of amphibian sympathetic ganglion cells. *J. Neurophysiol.* **31**, 109–121.

NISHI, S., SOEDA, H. & KOKETSU, K. (1965). Studies on sympathetic B and C neurons and patterns of preganglionic innervation. *J. cell. comp. Physiol.* **66**, 19–32.

NISHI, S., SOEDA, H. & KOKETSU, K. (1967). Release of acetylcholine from sympathetic preganglionic nerve terminals. *J. Neurophysiol.* **30**, 114–134.

OBRADOR, S. & ODORIZ, J. B. (1936). Transmission through a lumbar sympathetic ganglion. *J. Physicl.* **86**, 269-276.

PURVES, R. D. & BLACKMAN, J. G. (1968). Intracellular recordings from ganglia of the sympathetic chain of the guinea pig. *Proc. Univ. Otago med. Sch.* **46**, 44–47.

RUSHTON, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**, 101–122.

SKOK, V. I. (1963). Intracellular electric potentials of sympathetic ganglion neurons. *Fedn Proc.* **22**, T990–T993.

TOSAKA, T., CHICHIBU, S. & LIBET, B. (1968). Intracellular analysis of slow inhibitory and excitatory postsynaptic potentials in sympathetic ganglia of the frog. *J. Neurophysiol.* **31**, 396–409.